FILE COPY

U

SECURITY CLASSIFICATIC

AD-A226 200

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY C — ESTRICTIVE MARKINGS: NA

2a. SECURITY CLASSIFICA... — NA

3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited

2b. DECLASSIFICATION/DOWNGRAD... SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S): University of Florida

5. MONITORING ORGANIZATION REPORT NUMBER(S): NA

6a. NAME OF PERFORMING ORGANIZATION: University of Florida

6b. OFFICE SYMBOL (If applicable): NA

7a. NAME OF MONITORING ORGANIZATION: .Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code):
1059 McCarty Hall
University of Florida
Gainesville, Florida 32611

7b. ADDRESS (City, State, and ZIP Code):
800 N. Quincy Street
Arlington, VA 2217-5000

8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research

8b. OFFICE SYMBOL (If applicable): ONR

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-88-J-1047

8c. ADDRESS (City, State, and ZIP Code):
800 N. Quincy Street
Arlington, VA 2217-5000

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61153N	RR04106	441d016	

11. TITLE (Include Security Classification): (U) Biochemical-pathway diversity in Archaebacteria

12. PERSONAL AUTHOR(S): Jensen, Roy A.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 2-1-88 TO 6-30-90	1990/August/30	5

16. SUPPLEMENTARY NOTATION: NA

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Archaebacteria, biochemical diversity, prephenate dehydratase, aromatic amino acid biosynthesis.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Extreme halophiles such as Halobacterium vallismortis possess a prephenate dehydratase enzyme which is subject to allosteric activation by hydrophobic amino acids. This example of cross-pathway regulation (termed metabolic interlock) is characteristic of much or all of the Gram-positive lineage of eubacteria. We have extended the enzymological base of information to include organisms within the one of the three methanogen orders that is phylogenetically nearest to the Halobacteriales. Within the methanogen order, Methanomicrobiales, Methanohalophilus mahii (a member of the family Methanosarcinaceae) has been selected for in-depth study. The character states of aromatic amino acid biosynthesis have proven to be generally similar: in comparison of the extreme halophiles and the methanogen order studied here, differences were relatively minor, i.e., being of a quantitative nature rather than of a qualitative nature. In addition to the common possession of the interlock-type of prephenate dehydratase, enzymological similarities shared by extreme halophiles and Methanomicrobiales include the curious properties of chorismate mutase and the lack of detectable DAHP synthase (at moderate pH).

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: (U)

22a. NAME OF RESPONSIBLE INDIVIDUAL: M. Marron

22b. TELEPHONE (Include Area Code): (202) 696-4760

22c. OFFICE SYMBOL: ONR

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

DATE: 30 AUGUST 1990

FINAL REPORT ON CONTRACT N00014-8-J-1047 R&T CODE 441d016

PRINCIPAL INVESTIGATOR: Roy A. Jensen

CONTRACTOR: University of Florida

CONTRACT TITLE: Biochemical-pathway Diversity in Archaebacteria

START DATE: 1 February 1988

RESEARCH OBJECTIVE: To assess the extent to which the archaebacteria possess unique biochemical features of aromatic amino acid biosynthesis and regulation and to compare the biochemical diversity within the archaebacteria to the biochemical diversity already known or now emerging within the eubacteria.

RATIONALE: In eubacteria aromatic-pathway character states are exceedingly diverse. A given feature will cluster at a hierarchical level of phylogeny that cannot be predicted in advance, e.g., the bifunctional P-protein of phenylalanine synthesis is inevitably present in two of the three Superfamilies of Gram-negative bacteria, while the bifunctional T-protein of tyrosine synthesis evolved much later and is restricted to the enteric lineage (a relatively small cluster within Superfamily B). In eubacteria the type of prephenate dehydratase activated by hydrophobic amino acids has proven to be a character state conserved at a very deep level. Its presence in an extreme halophile identifies it as an enzyme of initial interest within the archaebacteria. Information can be anticipated at the level of overall aromatic amino acid biosynthesis (enzyme steps, cofactor specificity, regulatory patterns, multifunctional enzymes) that, although unpredictable a priori, will be interpretable in terms of evolution within the archaebacterial kingdom and in terms of deducing the nature of the pathway in a common ancestor of the three kingdoms.

INTRODUCTION: A distinct impression is popular that archaebacteria are biochemically unique. Thus, (i) methanogens possess several major cofactors that occur rarely, if at all, elsewhere, (ii) methanogens lack other cofactors which are ubiquitous elsewhere, (iii) archaebacteria exhibit unique cell-wall biochemistries, (iv) archaebacterial lipids are unique, (v) novel base modifications exist in tRNAs and rRNAs, and (vi) archaebacterial enzymes show unique subunit structure.

In eubacteria the comparative biochemistry and regulation of aromatic amino acid biosynthesis is known in a more comprehensive documentation of diversity than is any other biochemical pathway. Preliminary data from an extreme halophile indicate a productive approach to the

DTIC COPY INSPECTED

A-1

examination of biochemical diversity in archaebacteria that focuses upon the enzyme, prephenate dehydratase, as a starting point of inquiry.

PROGRESS: Extreme halophiles such as Halobacterium vallismortis possess a prephenate dehydratase enzyme which is subject to allosteric activation by hydrophobic amino acids. This example of metabolic interlock is characteristic of much or all of the Gram-positive lineage of eubacteria. We have extended the enzymological base of information in the extreme halophile lineage, and we have begun the study of organisms within the one of the three methanogen orders (Methanomicrobiales) that is phylogenetically nearest to the Halobacteriales. Within the latter methanogen order, Methanohalophilus mahii (a member of the family Methanosarcinaceae) has been selected for in-depth study.

Prephenate dehydrogenase. The enzyme exhibits a Km value for prephenate of 0.56 mM. It displays substrate ambiguity with respect to pyridine nucleotide requirement, although $NADP^+$ (Km = 0.079 mM) is preferred to NAD^+ (Km = 1.25 mM). It is quite sensitive to feedback inhibition by L-tyrosine, 100% inhibition being obtained at 0.2 mM L-tyrosine when Km levels of prephenate are used. Surprisingly, activity rates measured in the presence of 3.0 M KCl were 8-fold less than when 0.2 M KCl was present in the buffer.

Shikimate dehydrogenase. Like all eubacterial enzymes described to date, shikimate dehydrogenase was specific for $NADP^+$. It was incapable of substituting quinate for shikimate. Km values for shikimate and $NADP^+$ were 0.71 mM and 0.50 mM, respectively. Activity rates measured in the presence of 3.0 M KCl were 7-fold less than when 0.2 M KCl was present in the buffer.

Chorismate mutase. The enzyme is very active but possesses a rather low affinity for chorismate. Each of the three aromatic amino acids causes modest activation (in the range of 10%).

DAHP synthase. No activity was initially detected in spite of an extensive series of assays carried out under alternative conditions. Recent results indicate that the enzyme requires unusually high pH for activity, similar to plant DAHP synthases. Ongoing experiments can now determine the number of isozymes that may exist, together with the specialized pattern of allosteric control.

Other activities not detected. Arogenate dehydrogenase, arogenate dehydratase, and 4-hydroxyphenyllactate dehydrogenase (a new eubacterial enzyme of tyrosine biosynthesis) were not detected. These activities were not necessarily expected to be present.

Prephenate dehydratase. Activity was quite low in the absence of allosteric activators (tyrosine, tryptophan, leucine, methionine and isoleucine). The relative efficiencies of activator molecules were: TYR > LEU = MET > TRP > ILE. Activation by tyrosine was very dramatic

(13-fold at 2 mM TYR). Valine was ineffective as an activator. Phenylalanine was an exceedingly potent inhibitor, causing complete inhibition at only 13 μM. Phenylalanine was able to antagonize tyrosine activation quite effectively.

M. mahii may possess one of the most interesting prephenate dehydratases of the "metabolic interlock" class. Tyrosine activation of the M. mahii enzyme (13-fold) is much greater than the <2-fold effect seen with Halobacterium vallismortum, or the 21% increase seen with the Acholeplasma laidlawii enzyme. The cyanobacterial (Synechocystis) enzyme exhibits >6-fold activation by tyrosine but differs from M. mahii in the domination of activation over inhibition in Snyechocystis. Tryptophan activates the M. mahii enzyme substantially compared to its potent inhibitory effect in Bacillus subtilis.

ONGOING STUDIES: With the completion of enzymological characterizations at the biochemical level, molecular-genetic studies are now underway with chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase.

(i) Cloned genes encoding chorismate mutase (aroH), prephenate dehydratase (pheA) and prephenate dehydrogenase (tyrA) from Bacillus subtilis will be used to probe archebacterial gene banks. If a discriminating signal is observed, the gene bank can possibly be screened with a probe generated from full-length clones (note that heterologous probes from Streptomyces have been successfully used).

(ii) Oligonucleotides made to conserved domains determined from comparison of sequences of functionally related proteins are being prepared in order to probe a Methanohalophilus gene bank.

(iii) We will utilize the oligonucleotide probes to sequence the archebacterial genes encoding chorismate mutase, prephenate dehydratase, or prephenate dehydrogenase via application of PCR (polymerase chain reaction) methodology.

(iv) The cloned genes will be identified by functional complementation of existing Escherichia coli auxotrophs and/or auxotrophs to be constructed in Halobacterium.

Emerging perspective. The character states of aromatic amino acid biosynthesis are generally similar in the extreme halophiles and the methanogen order studied here. In addition to the common possession of the interlock-type of prephenate dehydratase, the enzymological similarities include the curious properties of chorismate mutase and the high-pH type of DAHP synthase. These results support the placement of extreme halophiles within the archaebacterial kingdom (as proposed by Woese), rather than in the eubacterial kingdom (as proposed by Lake).

PUBLICATIONS

Jensen, RA, TA d'Amato, & LI Hochstein. 1988. An extreme-halophile archaebacterium possesses the interlock type of prephenate dehydratase characteristic of the Gram-positive eubacteria. Arch. Microbiol. 148:365-371.

In addition a manuscript describing the overall pathway construction for aromatic amino acid biosynthesis and regulation in Methanohalophilus mahii is in preparation. A second manuscript describing molecular-genetic characterizations is anticipated.

ABSTRACTS

Jensen, RA. 1989. Gordon Conference on Population Biology and Evolution of Microorganisms. "Evolution of metabolic pathways". (Plymouth, New Hampshire).

Jensen, RA. 1989. Biochemical-pathway diversity in archaebacteria. ONR Archaebacteria Program Review Meeting. (Williamsburg, Virginia).

TRAINING ACTIVITIES:

Dr. Raj Bhatnagar, citizen of India
Dr. Tianhui Xia, citizen of China

INVENTIONS: None.